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## Analysis of bacterial spore permeability to water and ions using Nano-Secondary Ion Mass Spectrometry (NanoSIMS)

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Regulation of bacterial spore solvent and solute permeability is a fundamental feature of dormancy but is poorly understood. Here we present a new technique, nano-scale secondary ion mass spectrometry (NanoSIMS) that allows the direct visualization and quantification of chemical gradients within spores. Using NanoSIMS, we demonstrate the penetration of water and a simple ionic salt, LiF, into the core of *Bacillus thuringiensis israelensis* (*Bti*) spores. The results demonstrate chemical gradients spanning the outer coat to the inner spore core that are driven by concentration-dependent ionic fluxes. Using deuterated water (D<sub>2</sub>O), we have shown that external water is either retained or exchanged with water contained within the spore. Hydration and exchange are rapid, on a timescale of < 1 minute. Our results suggest a permeation mechanism by which short-time scale diffusion into and out of the spore can occur along hydration pathways. Additional studies are in progress to define the flux rates and mechanisms controlling these processes.

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